A CORRELATIVE MULTIMODAL IMAGING APPROACH FOR SPATIAL TRANSCRIPTOMICS MECHANOREGULATION ANALYSIS

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Introduction

Bone is a hierarchical tissue with intricate metabolic processes spanning several spatial scales. Consequently, we have recently developed a correlative multimodal imaging (CMI) approach to correlate *in vivo* 3D micro-computed tomography (micro-CT) images with *ex vivo* 2D histological sections from the same bone sample [1], enabling a holistic analysis of this multiscale system. Likewise, spatial transcriptomics (ST) has enabled measuring high-resolution spatially resolved expression of thousands of genes from histological sections. However, a link between sub-cellular activity and organ- and tissue-level data is still missing. Therefore, in this work, we aimed to expand our CMI approach [1] to register ST data to the corresponding 3D micro-CT image. Through micro-finite element analysis (micro-FE) on the 3D image, the local mechanical environment can be correlated with the gene expression measured, enabling a unique association of organ-to-cell events.

Methods

The data used in this work consisted of time-lapsed *in vivo* micro-CT scans (10.5 µm, vivaCT 80) and formalin-fixed paraffin-embedded (FFPE) histological sections (5 µm thickness) from an adult female mouse femur. Micro-CT scans were Gauss-filtered (sigma 1.2) and binarized at 395 mgHA/cm^3. A structurally intact FFPE section was prepared for the ST protocol [2] and transferred to a capture area of a ST slide (Visium, 10x Genomics). After probe hybridisation, ligation, library preparation and sequencing, the data was processed with Space Ranger (v.2.0.0). ST data was analysed with Scanpy and Squidpy, including Leiden clustering and uniform manifold approximation and projection (UMAP) algorithms. The 2D ST section was manually segmented based on the underlying tissues (bone, marrow, muscle, etc) and the mineralised bone matrix was 2D-3D registered to the corresponding *in vivo* micro-CT scan. Micro-FE analysis was performed to compute the mechanical signal as effective strain (EFF) using the 3D micro-CT image from the last time point. Spatially resolved gene expression data was correlated with the local mechanical environment (Spearman correlation coefficient, SCC).

Results

ST data yielded spatially resolved information for 19,112 genes (Q30 quality score above 93%, Figure 1A). Leiden clustering revealed anatomically credible clusters (Figure 1B) based on gene expression similarity, confirmed with UMAP. The ST data was successfully registered to the micro-CT scan (Figure 1C) and the mechanical environment was mapped to the gene counts obtained (Figure 1D), revealing genes both positively correlated with EFF, like Pick1 (SCC=0.26), and negatively correlated, such as Uqcrc2 (SCC=-0.33).

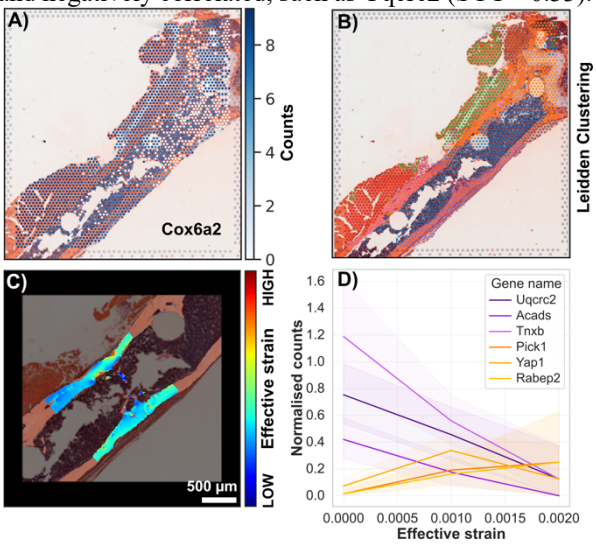

Figure 1: A) Representation of spatially resolved gene expression (Cox6a2). B) Output of unsupervised Leiden clustering. C) Mapping of 2D ST data with EFF from micro-FE. D) Mechanoregulation analysis of ST data, correlating EFF and normalized expression counts.

Discussion

This work advances previous results [1], by establishing the computational tools required for a complete multiscale investigation of biological processes in bone. It can be applied to explore bone regeneration in defect models (shown here) or bone adaptation with other established models (like mouse vertebral loading), highlighting how organ- and tissue-level events influence (sub-) cellular activity. Indeed, we identified potential mechanosensitive genes, whose expression is associated with the perceived local strain (Figure 1D). With more comprehensive ST datasets, we hope to solidify the gene expression trends observed and isolate suitable targets for improving healing outcome or counter degenerative conditions such as osteoporosis.

References

1. Marques et al., ESB Congress 2022
2. Wehrle et al. ORS Congress 2023, *accepted*

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